Assessment of the effect of Intermittent fasting in life traits of different species of *Drosophila*

Synopsis

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Jyoti Kumari 15/4/2024

Jyoti Kumari
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Under the Supervision of

Parul Banerjee 15/4/2024

Dr. Parul Banerjee
Assistant Professor,
University Department of Zoology,
LNMU, Darbhanga

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Introduction-

Intermittent fasting is meal timing schedule that cycles between fasting and non-fasting over a given period. Intermittent fasting include alternate-day fasting or periodic fasting or daily time-restricted eating. It has become popular as a weight loss regimen (Cabo and Mattson et al., 2019). Therefore, IF does not deal with alterations in dietary composition but controls the timing of feeding. Eating patterns that reduce or eliminate nighttime eating and prolong nightly fasting intervals may result in sustained improvements in human health. Intermittent fasting regimens are hypothesized to influence metabolic regulation via effects on (a) circadian biology, (b) the gut microbiome, and (c) modifiable lifestyle behaviors, such as sleep. If proven to be efficacious, these eating regimens offer promising non-pharmacological approaches to improving health at the population level, with multiple public health benefits (Patterson et al., 2017).

Importance of Intermittent fasting-

- Significant increase in Life span: Studies have shown that in *Drosophila, C. elegans*, and rodents time restricted fasting (TRF) leads to lifespan extension, the degree of which may vary between the sexes and the reproductive status (Cabrera et al., 2020, Hwangbo et al., 2020). Therefore, IF has been marked as a helpful pro-longevity intervention (Slack et al., 2011).
- Long-lasting stress resistance in *D. melanogaster*. IF in flies has an association with increased stress resistance (Slack et al., 2011), including starvation resistance, resistance to oxidative and xenobiotic stress etc. (Catterson et al., 2018).

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- Cells throughout the body and brain of animals maintained on intermittent fasting regimens have been shown to exhibit improved function and robust resistance to a broad range of potentially damaging insults, including those involving metabolic, oxidative, ionic, traumatic, and proteotoxic stress(Cabo and Mattson et al., 2019)..
- Weight loss: IF regimen has been linked to reduced fat mass and waist circumference, and lowered serum cholesterol, triglycerides, and C-reactive protein (Catterson et al., 2018). Alternate-day fasting in rodent models of obesity has also been shown to reduce total plasma cholesterol and triglyceride (TG) concentrations, reduce liver serosis and inflammatory gene expression, and have beneficial effects on cancer risk factors, such as cell proliferation (Gotthardt et al., 2016., Joslin et al., 2017, Varady and Hellerste, 2007, Yang et al., 2016).

Models suitable for studying the effect of IF-

Nematode *(Caenorhabditis elegans)*: *C. elegans* has also turned out to be the model of choice. It has basically been used for aging studies. Having a relatively short lifespan/reproductive cycle and a translucent body, it is easy to culture. Also, it has a small genome, and there are many available mutants. IF regimen (alternate 2 days eating/ 2 days fasting) has been found to successfully extended lifespan in the worms (Hwangbo et al 2020). Furthermore, chronic fasting also increased the lifespan of worms compared to normal diet-fed worms.

Fruit Fly *(Drosophila melanogaster)*: The fruit fly *Drosophila* has several qualities like ease in rearing/handling/ crossing, short generation time etc. that makes it a model of choice for varied types of studies employing some ingenious genetical and biochemical tools. For example, *D. melanogaster*, has been widely used for aging and dietary intervention studies. Gene manipulation and editing tools are also readily

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available to study the genes of interest in a time- and tissue-controlled manner (Piper et al., 2018). IF has been found to increase the lifespan of fruit flies (Hwangbo et al., 2020). However, a 3 h or 6 h starvation during the day was not enough to extend the lifespan. Additionally, TRF was not found to increase the median lifespan of fruit flies, although TRF improved the muscle performance and attenuated age-related cardiac dysfunction (Longoet al. 2016 and Villanueva et al., 2018).

Further, IF has also been found to prolong life span, reverse the process of aging, increase stress resistance, preserve gut homeostasis, reduce fecundity (Catterson et al., 2018), reduce macromolecule concentration, maintain circadian rhythm (Cabo and Mattson,2019).

Rodents: In several studies Time Restricted fasting (TRF) has been shown to inhibit several chronic diseases and tumor progression leading to increased lifespan in rodents (Hwangbo et al., 2020).

Mechanisms through which IF might work-

Autophagy-

Autophagy refers to the process by which cells break down and destroys old, damaged or abnormal proteins and other substances in its own cytoplasm, through the formation of autophagosomes. This is required for recycling of cellular products. It has been dubbed as a natural cleansing out process. Researchers are exploring the role of autophagy in fighting and preventing diseases. IF may exhibit its effect through autophagy (Khandia et al., 2019).

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Circadian Clock-

Circadian clock regulate the timing of physiological functions. Nutrient and energy metabolism in mammals exhibits strong diurnal rhythm that aligns with the body clock. Nutrient and energy metabolism is temporally organized in mammalian tissues to synchronize the storage and utilization of energy with light/dark cycles. Circulating metabolites and hormones ebb and flow according to distinct diurnal patterns (Ma, D. et al., 2012).

IF and circadian rhythm

Most animals alternate periods of feeding with periods of fasting often coinciding with sleep. Upon >24 hr of fasting, humans, rodents, and other mammals have been found to enter alternative metabolic phases, which rely less on glucose and more on ketone body-like carbon sources. Both intermittent and periodic fasting has been found to modulate the 'clock' to improve metabolism (longo et al., 2014, 2016). Similarly, time-restricted feeding (TRF), in which food consumption is restricted to certain hours of the day, allows the daily fasting period to last >12 hr, thus imparting pleiotropic benefits (Longo et al., 2016).

Circadian regulation and autophagy-

Both circadian regulation and autophagy are highly conserved processes in human. This opens up the possibility that interventions particularly those involving modulations in meal timings would influence circadian-regulated autophagy and provide individuals with health benefits, such as delayed ageing and lifespan extension (Cabo and Mattson et al., 2019).

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The Crux-

Although extensive studies have been done on Intermittent Fasting (IF) in various model organisms, the exact way by which IF might be affecting different species or whether it has the same effect on different species is still not clear. To corroborate the degree of influence of IF, its effect should be tested in a number of species together keeping the other sets of conditions constant. With this in view, the present study will be undertaken to unearth the effects of IF on different species of *Drosophila* and predict its sway in humans.

Aim-

To compare the effects of Intermittent fasting in different species of *Drosophila.*

Objectives-

1. Effect of Intermittent fasting in different age groups and comparison across different species.

2. Effect of short v/s long term Intermittent fasting on different species *of Drosophila.*

3. Effect of Intermittent fasting with and without dietary restriction in different species of *Drosophila.*

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Methodology-

Locally available *Drosophila* species will be collected for experimental purpose. Species identification will be done with the help of key.

1. To track the effect of Intermittent fasting in different age groups, virgin females and bachelor males will be collected and aged for different periods, not extending seven days before starting IF regimen. The following schedule will be followed:

a) 24 hour unrestricted access to food (control)

b) 12 hrs day time access to food

c) 24 hours fasting followed by 24 hrs unrestricted feeding.

Each age group of flies will be given IF treatment (in all the regimens) for at least 3 days before beginning to test their effect. The following assays will be done on males and females to test the effect of intermittent fasting:

- Longevity
- Mating propensity

In females, additionally, the following assays will be done:

- Fecundity
- Fertility

The results obtained in different species of flies will be compared through suitable statistical analysis to check if IF in different age groups has the same effect on different species.

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2. Effect of short term v/s long term Intermittent fasting:

Virgin males and females will be collected and divided into different groups. The different groups will be aged for different number of days as follows:

- 2 days
- 4 days
- 6 days

Each group will then further be divided into groups and subjected to short v/s long term IF. Short term IF will be for 2 days and long term for 10 days.

The short and long term IF treated flies will be tested for longevity.

The results obtained in different species of flies will be compared through suitable statistical analysis to check if short term v/s long term IF affects different species differently.

3. Effect of intermittent fasting with and without dietary restriction in different species of *Drosophila.*

The first and second sets of experiments will be repeated with dietary restriction (DR) to check if the effects are the same. The IF group alone and IF plus DR groups will be compared through suitable statistical tests across different species.

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Expected Result

This study is expected to give novel insights into the effect of intermittent fasting in different life traits of *Drosophila*. While IF has been hailed as a potent weight loss regimen, its species specific effects in life history traits may throw novel insights into its precise role. Therefore, this study is expected to throw more light on the effect of IF.

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Submitted by

Jyoti Kumari 15/4/2024

Jyoti Kumari
Pat Roll No. E2221006
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Under the Supervision of

Parul B 15/4/2024

Dr. Parul Banerjee
Assistant Professor,
University Department of Zoology,
LNMU, Darbhanga